

FCS10 – SOP for Chemical Spot Tests

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1. Scope

- 1.1. To describe the chemical screening procedures, commonly referred to as chemical spot tests, for preliminary or screening tests of controlled and non-controlled substances.

2. Background

- 2.1. To establish the practices for documenting the examination of evidence to conform to the requirements of the Department of Forensic Sciences (DFS) Forensic Science Laboratory (FSL) *Quality Assurance Manual*, the accreditation standards under ISO/IEC 17025 (current version), and supplemental standards.

3. Safety

- 3.1. Read Material Safety Data Sheets to determine the safety hazards for chemicals and reagents used in the standard operating procedures.
- 3.2. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures.
- 3.3. Note: when handling items that are suspected to contain fentanyl, or fentanyl analogs, accessioning in fume hood is advised when possible. If the substance is

powder or has the potential for aerosolization, then the item shall be accessioned and sampled in the fume hood.

4. Materials Required

- 4.1. Spot plates, pipettes, or other appropriate containers/items
- 4.2. Reagents appropriate to the specific chemical spot tests

5. Standards and Controls

- 5.1. Each reagent must be labeled with the name of the solution or reagent. The analyst's initials and the date prepared must be recorded on the label or in an appropriate logbook.
- 5.2. Freshly prepared reagents will be quality tested with known reference standards and the results recorded in a retrievable logbook. Reagents will be tested by someone other than the preparer.
- 5.3. Unless otherwise specified, performance of reagents will be verified quarterly, and the results of the checks placed in a logbook. If the reagent has not been used for a month or more, it must be checked using a standard (and the results of the check logged) before its use with case samples.
- 5.4. It is the responsibility of the analyst to determine if reagents are working properly, and to periodically quality-test them and document the results. Reagents which do not respond appropriately to quality testing will be discarded.
- 5.5. Each set of color test performed will be accompanied with a corresponding blank.

6. Calibration

- 6.1. Not applicable.

7. Procedures

- 7.1. General Procedures for Preparing Color Test Reagents
 - 7.1.1. The method of preparation for all laboratory-prepared chemical reagents shall be recorded in a laboratory notebook, or electronic equivalent.
 - 7.1.2. Any chemical reagent produced within the laboratory shall follow a documented formulation.

- 7.1.3. Documentation for reagents prepared within the laboratory shall include identity, concentration (when appropriate), date of preparation, identity of the individual preparing the reagents, storage conditions (if appropriate), and the expiration date.
- 7.1.4. A reagent logbook will be maintained to document reagent preparation, testing, and retesting.
- 7.1.5. If any reagents fail testing, all reagents from the same lot will be disposed of immediately.
- 7.1.6. Chemists will ensure that all their reagents are not expired and have been tested in the last quarterly test before using the reagents in casework.
- 7.1.7. Each color test will be assigned a unique lot number when it is created (or when opened, if it is manufacturer prepared).
- 7.1.8. For in house preparations, or manufacturer prepared reagents that do not already possess a lot number, the following format shall be used:
 - 7.1.8.1. Initialism of the color test used, e.g.,
 - 7.1.8.1.1. CT for Cobalt Thiocyanate
 - 7.1.8.1.2. MQ for Marquis Reagent
 - 7.1.8.1.3. DL for Duquenois-Levine Reagent
 - 7.1.8.1.4. MY for Mayer's Reagent
 - 7.1.8.1.5. FC for Ferric Chloride
 - 7.1.8.1.6. ER for Ehrlich's Reagent
 - 7.1.8.1.7. SN for Sodium Nitroprusside
 - 7.1.8.2. Date of preparation (e.g., YYYYMMDD format)
 - 7.1.8.3. Preparer's initials (or MP if manufacturer prepared)
 - 7.1.8.4. Example of a lot number for Brandon Paul Jones preparing a Marquis Reagent on April 2, 2018, would be MQ20180402BPJ.

7.1.9. For manufacturer prepared reagents that possess a lot number, the following format shall be used:

7.1.9.1. Manufacturer's assigned lot number followed by MP (manufacturer prepared).

7.1.9.2. Example of a lot number for a purchased bottle of Mayer's reagent would be 4201D77MP.

7.1.10. Reagents used for color tests will be tested prior to use (by someone other than the preparer for reagents prepared within the laboratory).

7.1.11. Each lot of color test reagents will be retested quarterly or more often, if necessary.

7.1.12. Expiration dates for color test reagents prepared within the laboratory will be set as 3 months from the last quarterly test (or from the preparation date if no additional quarterly retests have been performed).

7.1.13. Expiration dates for manufacturer prepared color test reagents will be set for the manufacturer's provided expiration date.

7.1.14. If no expiration date is provided by the manufacturer, the expiration date will follow the laboratory prepared color test reagent expiration date guidelines.

7.2 MARQUIS TEST

7.2.1 Reagents/Chemicals:

7.2.1.1 Concentrated Sulfuric Acid

7.2.1.2 Formaldehyde Solution (approx. 37% Formaldehyde)

7.2.2 Reagent Preparation:

7.2.2.1 Marquis Reagent: Add 5 to 10mL of approx. 40% formaldehyde solution to 100mL of concentrated sulfuric acid

7.2.2.2 Store in a cool, dry place

7.2.2.3 Quality-test reagent with heroin or acetylsalicylic acid

7.2.3 Procedure:

7.2.3.1 Add a couple of drops of reagent to a test container

7.2.3.2 Add a small amount of sample to test container containing Marquis reagent

7.2.3.3 Observe and record any color change(s).

7.2.3.4 **Note:** If during the period of observation multiple color transitions are observed, the Analyst shall record all relevant colors observed, as determined by the Analyst.

7.2.4 Interpretation (color test acceptance criteria):

7.2.4.1 **Purple** may indicate presence of Heroin

7.2.4.2 **Orange to Brown** may indicate presence of Methamphetamine

7.2.4.3 **Yellow to Brown to Violet** may indicate presence of Oxycodone

7.2.4.4 **Orange to Brown** may indicate presence of Amphetamine

7.2.4.5 **Pink/Red** may indicate presence of Acetylsalicylic Acid

7.2.4.6 **Salmon** may indicate presence of Cocaine

7.2.4.7 **Note:** This is not an exhaustive list of possible results. A “positive”, +, result is considered to be any color change that is expected, as indicated by a credible reference (i.e., UNODC – “Rapid Testing Methods of Drugs of Abuse”, Johns, S. H. et. al. – “Spot Tests: A Color Chart Reference for Forensic Chemists”, or other peer reviewed literature).

7.2.4.8 The color(s) which appear(s) must be documented on the examination worksheet. For example, if during the period of observation multiple color transitions are observed, the Analyst shall record all relevant colors observed, as determined by the Analyst.

7.3 COBALT THIOCYANATE

7.3.1 Reagents/Chemicals:

7.3.1.1 Cobalt Thiocyanate

7.3.1.2 Deionized or higher quality water (H₂O)

7.3.1.3 Dilute Hydrochloric Acid (HCl), acetic acid, or other acid

7.3.2 Reagent Preparation:

7.3.2.1 2% Cobalt Thiocyanate Reagent: Dissolve 2g cobalt thiocyanate in 100mL water (deionized grade or higher quality)

7.3.2.2 Store in a cool, dry place

7.3.2.3 Quality-test reagent with procaine hydrochloride or cocaine standard

7.3.3 Procedure:

7.3.3.1 Add a couple drops of reagent to a test container

7.3.3.2 Add a small amount of sample to test container containing Cobalt Thiocyanate reagent (pink color)

7.3.3.3 Observe and record any color change(s)

7.3.3.4 **Note:** If a positive result is obtained (pink turns to blue color), the analyst may stop and record any observations.

7.3.3.5 **Note:** If the color remains pink, add a few drops of acetic acid to the sample and record any observations.

7.3.4 Interpretation (color test acceptance criteria):

7.3.4.1 Upon mixing sample with reagent, a **blue color results** (positive, +, result), indicating cocaine, or another secondary or tertiary amine, may be present.

7.3.4.2 **Note:** A pink to blue color change that occurs only after the addition of acetic acid may indicate the presence of cocaine base.

7.3.4.3 The color(s) which appear(s) must be documented on the examination worksheet.

7.3.4.4 **Note:** Known False Positives – Acetonitrile gives a positive (blue) result upon addition of cobalt thiocyanate. Care should be taken to ensure that neat samples are tested (or samples extracted in other solvents) and not acetonitrile extracts to avoid false positive results.

7.4 SODIUM NITROPRUSSIDE TEST

7.4.1 Reagents/Chemicals:

- 7.4.1.1 Sodium Nitroprusside
- 7.4.1.2 Purified H₂O
- 7.4.1.3 Acetaldehyde
- 7.4.1.4 1M Sodium Hydroxide (NaOH)
- 7.4.1.5 1M Sodium Carbonate (Na₂CO₃)

7.4.2 Reagent Preparation:

- 7.4.2.1 Sodium Nitroprusside Reagent: Dissolve 0.09g sodium nitroprusside in a mixture of 1mL acetaldehyde and 9mL water.
- 7.4.2.2 Store in a cool, dry place
- 7.4.2.3 Quality-test reagent with a methamphetamine standard.

7.4.3 Procedure:

- 7.4.3.1 Add a couple drops of reagent to test container
- 7.4.3.2 Add a small amount of sample to test container containing Sodium Nitroprusside reagent
- 7.4.3.3 Add a few drops of 1M Na₂CO₃ (or NaOH) to the sample
- 7.4.3.4 Observe and record any color change(s)

7.4.4 Interpretation (color test acceptance criteria):

- 7.4.4.1 Upon mixing sample with reagent, a **blue color results** (positive, +, result), indicating the possible presence of secondary amines, such as methamphetamine.
- 7.4.4.2 The color(s) which appear(s) must be documented on the examination worksheet

7.5 FERRIC CHLORIDE TEST

7.5.1 Reagents/Chemicals:

7.5.1.1 Ferric Chloride, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$

7.5.1.2 Deionized water or higher grade (H_2O)

7.5.2 Reagent Preparation:

7.5.2.1 5% Ferric Chloride Reagent: Dissolve 0.83g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 10mL water

7.5.2.2 Store in a cool, dry place

7.5.2.3 Quality-test reagent with gamma-hydroxybutyrate (GHB) or aspirin.

7.5.3 Procedure:

7.5.3.1 Add a couple drops of reagent to a test container

7.5.3.2 Add a small amount of sample to test container containing 5% Ferric Chloride reagent

7.5.3.3 Observe and record any color change(s)

7.5.4 Interpretation (color test acceptance criteria):

7.5.4.1 **Orange/brown** may indicate the presence of GHB.

7.5.4.2 **Dark purple** may indicate the presence of salicylates.

7.5.4.3 **Bluish-gray** may indicate the presence of acetaminophen.

7.5.4.4 The color(s) which appear(s) must be documented on the examination worksheet.

7.6 COBALT NITRATE TEST

7.6.1 Reagents/Chemicals

7.6.1.1 Cobalt nitrate

7.6.1.2 Isopropylamine

7.6.1.3 95% Ethanol

7.6.2 Reagent Preparation:

7.6.2.1 1% Cobalt nitrate in ethanol reagent: Add 1g cobalt nitrate to 100mL ethanol

7.6.2.2 5% Isopropylamine in ethanol reagent: Add 5g isopropylamine to 100mL ethanol

7.6.2.3 Store in a cool, dry place

7.6.2.4 Quality-test reagent with a gamma-hydroxybutyrate (GHB) or barbiturate standard.

7.6.3 Procedure:

7.6.3.1 Add a couple drops of 1 % cobalt nitrate reagent to a test container

7.6.3.2 Add a small amount of sample to test container containing 1% cobalt nitrate in ethanol reagent

7.6.3.3 Observe and record any color changes(s)

7.6.3.4 Add a few drops 5% isopropylamine reagent to sample

7.6.3.5 Observe and record any color changes(s)

7.6.4 Interpretation (color test acceptance criteria)

7.6.4.1 **Purple color** (upon addition of 1% cobalt nitrate in ethanol) may indicate the presence of GHB.

7.6.4.2 **Purple color** (which only forms after also adding 5% isopropylamine in ethanol) may indicate the presence of barbiturates.

7.6.4.3 The color(s) which appear(s) must be documented on the examination worksheet

7.7 p-DMABA TEST

7.7.1 Reagents/Chemicals:

7.7.1.1 95% Ethanol

7.7.1.2 p-Dimethylaminobenzaldehyde

7.7.1.3 Concentrated Hydrochloric Acid (HCl)

7.7.2 Reagent Preparation:

7.7.2.1 p-DMABA reagent: Dissolve 0.1g p-dimethylaminobenzaldehyde in 9.5mL ethanol. Add 0.5 ml concentrated HCl

7.7.2.2 Store in a cool, dry place

7.7.2.3 Quality-test reagent with benzocaine, procaine, or lysergic acid diethylamide (LSD)

7.7.3 Procedure:

7.7.3.1 Add a couple drops of reagent to a test container

7.7.3.2 Add a small amount of sample to test container containing p-DMABA reagent

7.7.3.3 Observe and record any color change(s)

7.7.4 Interpretation (color test acceptance criteria):

7.7.4.1 **Bright yellow** may indicate the possible presence of procaine or benzocaine

7.7.4.2 **Purple** may indicate the possible presence of LSD

7.7.4.3 The color(s) which appear(s) must be documented on the examination worksheet

7.8 WEBER TEST

7.8.1 Reagents/Chemicals:

7.8.1.1 Fast Blue B (o-Dianisidine bis(diazotized) zinc double salt)

7.8.1.2 Concentrated Hydrochloric Acid (HCl)

7.8.1.3 Deionized water, or higher quality (H₂O)

7.8.2 Reagent Preparation:

7.8.2.1 0.1% Fast Blue B: Dissolve 0.1g Fast Blue B in 100mL H₂O

7.8.2.2 Store in a cool, dry place

7.8.2.3 Prepare reagent fresh and quality test with psilocin or mushroom sample known to contain psilocin before use

7.8.3 Procedure:

7.8.3.1 Add a couple drops of reagent to a test container

7.8.3.2 Add a small amount of sample or methanol extract of the sample to test container containing 0.1% Fast Blue B. Wait approximately one minute.

7.8.3.3 Add a couple of drops of concentrated HCl

7.8.3.4 Observe and record any color change(s)

7.8.4 Interpretation (color test acceptance criteria):

7.8.4.1 **Red** (after addition of Fast Blue B reagent) with a change to blue (after addition of HCl) may indicate the presence of Psilocin (appropriate color sequence = Weber +)

7.8.4.2 The color(s) which appear(s) must be documented on the examination worksheet

7.9 DUQUENOIS-LEVINE TEST (Modified)

7.9.1 Reagents/Chemicals:

7.9.1.1 Vanillin

7.9.1.2 95% Ethanol

7.9.1.3 Acetaldehyde

7.9.1.4 Concentrated Hydrochloric Acid (HCl)

7.9.1.5 Chloroform

7.9.1.6 Petroleum Ether

7.9.2 Reagent Preparation:

7.9.2.1 Duquenois Reagent: Add 2g Vanillin, 2 mL Acetaldehyde, and 100mL 95% Ethanol to bottle (>100mL size)

7.9.2.2 Store in a cool, dry place

7.9.2.3 Quality-test reagent with Marijuana or Delta-9-Tetrahydrocannabinol (Δ^9 -THC).

7.9.3 Procedure:

7.9.3.1 Place small amount of plant material in testing container. Either proceed directly to next step or extract plant material with petroleum ether. Discard plant material and evaporate to dryness. This step is optional and may be performed on difficult samples or at the discretion of the analyst.

7.9.3.2 Add approximately one milliliter of the Duquenois reagent.

7.9.3.3 Add approximately one milliliter of concentrated Hydrochloric Acid and wait up to a minute for color to appear. (It is not necessary to wait as long with the extract.)

7.9.3.4 Observe and record any color change(s).

7.9.3.5 Add approximately two milliliters of chloroform and wait for layers to form.

7.9.3.6 Observe and record any color change(s) and layers.

7.9.4 Interpretation (color test acceptance criteria):

7.9.4.1 **Blue to violet** color (after addition of HCl) may indicate the presences of cannabinoids (positive reaction).

7.9.4.2 **Purple** in organic (lower) layer may indicate the presences of cannabinoids (positive reaction).

7.9.4.3 A positive result indicates that components (cannabinoids, including THC) unique to marijuana, marijuana residue, or hashish may be present.

7.9.4.4 A positive (or +) indication on the worksheet means test resulted in a purple/violet color after addition of hydrochloric acid and Duquenois reagent, and that the chloroform layer also yields purple color.

7.9.4.5 The color(s) which appear(s) must be documented on the examination worksheet.

7.9.4.6 **Note:** Known False Positives: Plants patchouli, cypress, and eucalyptus

7.10 FORMALDEHYDE-SULFURIC ACID TEST

7.10.1 Reagents/Chemicals:

7.10.1.1 Concentrated Sulfuric Acid

7.10.1.2 Formaldehyde Solution (*i.e.*, 37% Formaldehyde)

7.10.2 Reagent Preparation:

7.10.2.1 Formaldehyde-Sulfuric Reagent: Add 6mL of formaldehyde solution to 4mL Concentrated Sulfuric acid. Keep the pipette tip just below the surface during the addition; stir and (if necessary) cool the mixture.

7.10.2.2 Store in a cool, dry place.

7.10.2.3 Quality-test reagent with a known sample of benzodiazepines.

7.10.3 Procedure:

7.10.3.1 Add a couple drops of reagent to test container

7.10.3.2 Add a small amount of sample to test container containing formaldehyde-sulfuric test reagent

7.10.3.3 Heat to approximately 100°C for approximately a minute.

7.10.3.4 Observe and record any color change(s).

7.10.4 Interpretation (color test acceptance criteria):

7.10.4.1 **Orange** may indicate the presence of benzodiazepines

7.10.4.2 The color(s) which appear(s) must be documented on the examination worksheet

7.11 JANOVSky TEST

7.11.1 Reagents/Chemicals:

7.11.1.1 m-dinitrobenzene

7.11.1.2 Potassium hydroxide

7.11.1.3 Absolute ethanol

7.11.2 Reagent Preparation:

7.11.2.1 Janovsky Solution A (2% m-dinitrobenzene): Add 2g m-dinitrobenzene to 100mL absolute ethanol

7.11.2.2 Janovsky Solution B (5N potassium hydroxide): Add 28.05g potassium hydroxide to 100mL purified

7.11.2.3 Store in a cool, dry place

7.11.2.4 Quality-test reagents with Ketamine and/or Flunitrazepam

7.11.3 Procedure:

7.11.3.1 Add equal amounts of Janovsky Solution A and B reagents to test container

7.11.3.2 Add a small amount of sample to test container containing reagents

7.11.3.3 Observe and record any color change(s)

7.11.4 Interpretation (color test acceptance criteria):

7.11.4.1 **Brown/purple with purple precipitate/specks** may indicate the presence of Ketamine. The purple color will intensify with heat and time

7.11.4.2 **Strong purple that fades to brown** may indicate the presence of Flunitrazepam

7.11.4.3 The color(s) which appear(s) must be documented on the examination worksheet

7.12 EHRLICH TEST

7.12.1 Reagents/Chemicals:

- 7.12.1.1 p-dimethylaminobenzaldehyde
- 7.12.1.2 Distilled water
- 7.12.1.3 Concentrated Hydrochloric Acid (HCl)

7.12.2 Reagent Preparation:

- 7.12.2.1 Ehrlich Reagent: Dissolve 1g of p-dimethylaminobenzaldehyde to 100mL distilled water. Add 10mL concentrated hydrochloric acid.
- 7.12.2.2 Store in a cool, dry place
- 7.12.2.3 Quality-test reagents with psilocybin and/or psilocin

7.12.3 Procedure:

- 7.12.3.1 Add a couple drops of reagent to a test container
- 7.12.3.2 Add a small amount of sample to test container containing Ehrlich reagent
- 7.12.3.3 Observe and record any color change(s)

7.12.4 Interpretation (color test acceptance criteria):

- 7.12.4.1 **Violet** may indicate the presence of psilocybin, psilocin, or LSD
- 7.12.4.2 The color(s) which appear(s) must be documented on the examination worksheet

7.13 MAYER'S TEST

7.13.1 Reagents/Chemicals:

- 7.13.1.1 Mercuric chloride
- 7.13.1.2 Distilled water
- 7.13.1.3 Potassium Iodide

7.13.1.4 10% Hydrochloric Acid (or acetic acid)

7.13.2 Reagent Preparation:

7.13.2.1 Mayer's Reagent: Dissolve 1g of mercuric chloride in 100mL distilled water. Add potassium iodide to dissolve scarlet precipitate.

7.13.2.2 Store in a cool, dry place

7.13.2.3 Quality-test reagents with Phencyclidine (PCP) or Cocaine standard

7.13.3 Procedure:

7.13.3.1 Add a couple drops of reagent to test container

7.13.3.2 Add a small amount of sample to test container containing Mayer's reagent

7.13.3.3 Add approximately 0.5mL 10% HCl or acetic acid

7.13.3.4 Observe and record any color change(s)

7.13.4 Interpretation (color test acceptance criteria):

7.13.4.1 **White precipitate formation** may indicate the presence of a tertiary amine.

7.13.4.2 The color(s) which appear(s) must be documented on the examination worksheet

8. Sampling

8.1. Not applicable

9. Calculations

9.1. Not applicable

10. Uncertainty of Measurement

10.1. Not applicable

11. Limitations

- 11.1. All spot tests are presumptive in nature and serve only as a guide for an analyst's analytical scheme.
- 11.2. Adulterants and complex mixtures may produce reactions that interfere with the interpretations.

12. Documentation

- 12.1. FCU Examination Worksheets
- 12.2. FCU Laboratory Report

13. References

- 13.1. Forensic Science Laboratory Quality Assurance Manual (Current Version)
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